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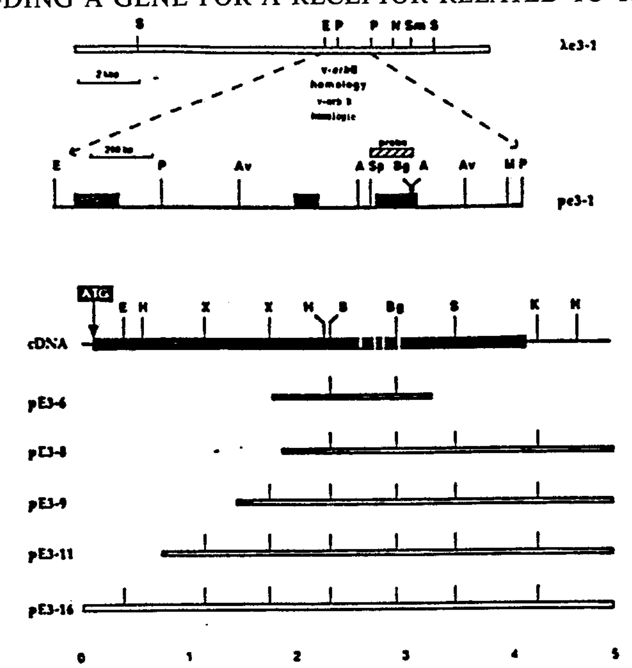

(57) Abstract

A DNA fragment distinct from the epidermal growth factor receptor (EGF-R) and *erb*B-2 genes was detected by reduced stringency hybridization of v-*erb*B to normal genomic human DNA. Characterization of the cloned DNA fragment mapped the region of v-*erb*B homology to three exons with closest homology of 64 % and 67 % to a contiguous region within the tyrosine kinase domains of theEGF-R and *erb*B-2 proteins, respectively. cDNA cloning revealed a predicted 148 kd transmembrane polypeptide with structural features identifying it as a member of the *erb*B family, prompting designation of the new gene as *erb*B-3. It was mapped to human chromosome 12q11-13 and was shown to be expressed as 6.2 kb transcript in a variety of normal tissues of epithelial origin. Markedly elevated *erb*B-3 mRNA levels were demonstrated in certain human mammary tumor cell lines. These findings indicate that increased *erb*B-3 expression, as in the case of EGF-R and *erb*B-2, plays a role in some human malignancies.

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DNA SEGMENT ENCODING A GENE FOR A RECEPTOR RELATED TO THE EPIDERMAL GROWTH FACTOR RECEPTOR

FIELD OF THE INVENTION

The present invention relates to genes which encode novel proteins related to a family of receptor proteins typified by two related membrane scanning tyrosine kinases: the Epidermal Growth Factor receptor (EGF-R), which is encoded by the *erb*B gene, the normal human counterpart of an oncogene (v-*erb*B) that was first recognized in the proviral DNA of avian erythroblastosis virus; and the receptor encoded by the related gene *erb*B-2. In particular, the present invention relates to a DNA segment encoding the coding sequence, or a unique portion thereof, for a third member of this receptor gene family, herein designated *erb*B-3.

BACKGROUND OF THE INVENTION

Proto-oncogenes encoding growth factor receptors constitute several distinct families with close overall structural homology. The highest degree of homology is observed in their catalytic domains, essential for the intrinsic tyrosine kinase activity of these proteins. Examples of such receptor families include: the EGF-R and the related product of the *erb*B-2 oncogene; the Colony Stimulating Factor 1 receptor (CSF-1-R) and the related Platelet-Derived Growth Factor receptor (PDGF-R); the insulin receptor (IF-R) and the related Insulin-like Growth factor 1 receptor (IGF-1-R); and the receptors encoded by the related oncogenes *eph* and *elk*.

It is well established that growth factor receptors in several of these families play critical roles in regulation of normal growth and development. Recent studies in *Drosophila* have emphasized how critical and multifunctional are developmental processes mediated by ligand-receptor interactions. An increasing number of *Drosophila* mutants with often varying phenotypes have now been identified as being due to lesions in genes encoding such proteins. The genetic locus of the *Drosophila* EGF-R homologue, designated DER, has recently been identified as

being allelic to the zygotic embryonic lethal *faint little ball* exhibiting a complex phenotype with deterioration of multiple tissue components of ectodermal origin. Furthermore, other mutants appear to lack DER function either in the egg or the surrounding maternal tissue. Thus, the DER receptor may play an important role in the ligand-receptor interaction between egg and follicle cells necessary for determination of correct shape of eggshell and embryo. It is not yet known whether DER represents the sole of the *Drosophila* counterpart of both known mammalian *erb*B-related genes.

Some of these receptor molecules have been implicated in the neoplastic process as well. In particular, both the *erb*B and *erb*B-2 genes have been shown to be activated as oncogenes by mechanisms involving overexpression or mutations that constitutively activate the catalytic activity of their encoded receptor proteins (Bargmann, C. I., Hung, M. C. & Weinberg, R. A., 1986, *Cell* 45:649-657; Di Fiore, P. P., Pierce, J. H., Kraus, M. H., Segatto, O., King, C. R. & Aaronson, S. A., 1987, *Science* 237:178-182; Di Fiore, P. P., Pierce, J. H., Fleming, T. P., Hazan, R., Ullrich, A., King, C. R., Schlessinger, J. & Aaronson, S. A., 1987, *Cell* 51:1063-1070; Velu, T. J., Beguinot, L., Vass, W. C., Willingham, M. C., Merlino, G. T., Pastan, I. & Lowy, D. R., 1987, *Science* 238:1408-1410). Both *erb*B and *erb*B-2 have been casually implicated in human malignancy. *erb*B gene amplification or overexpression, or a combination of both, has been demonstrated in squamous cell carcinomas and glioblastomas (Libermann, T. A., Nusbaum, H. R., Razon, N., Kris, R., Lax, I., Soreq, H., Whittle, N., Waterfield, M. D., Ullrich, A. & Schlessinger, J., 1985, *Nature* 313:144-147). *erb*B-2 amplification and overexpression have been observed in human breast and ovarian carcinomas (King, C. R., Kraus, M. H. & Aaronson, S. A., 1985, *Science* 229:974-976; Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A. & Press, M. F.,

1989, *Science* 244:707-712), and *erb*B-2 overexpression has been reported to be an important prognostic indicator of particularly aggressive tumors (Slamon, D. J., et al., 1989, *supra*). Yet, not all such tumors have been found to overexpress *erb*B-2, and many human tumors have not yet been associated with any known oncogene. Thus, there has been a continuing need to search for additional oncogenes which would provide knowledge and methods for diagnosis and, ultimately, for rational molecular therapy of human cancers.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a DNA segment encoding a receptor protein related to the *erb*B proto-oncogene family which previously has not been known or even suspected to exist. Further, it is an object of the present invention to develop assays for expression of the RNA and protein products of such genes to enable determining whether abnormal expression of such genes is involved in human cancers.

In pursuit of the above objects, the present inventors have discovered a human genomic DNA fragment that is produced by cleavage with the *Sac*I restriction enzyme, has a size of about 9 kbp, and is detectable by nucleic acid hybridization with a probe derived from the v-*erb*B gene only under reduced stringency hybridization conditions. Thus, this DNA fragment is distinct from those known to encode the epidermal growth factor receptor (EGF-R) (i.e., the *erb*B gene) and from the related *erb*B-2 gene. Characterization of this DNA fragment after partial purification and molecular cloning showed that the region of v-*erb*B homology mapped to three exons that encode amino acid sequences having homologies of 64% and 67% to contiguous regions within the tyrosine kinase domains of the EGF-R and *erb*B-2 proteins, respectively. A probe derived from the genomic DNA clone identified cDNA clones of the related mRNA which encode a predicted 148 kd transmembrane polypeptide with structural features identifying it as a member of the *erb*B family, prompting designation of the

new gene as *erb*B-3. This gene was mapped to human chromosome 12q11-13 and was shown to be expressed as a 6.2 kb transcript in a variety of normal tissues of epithelial origin. Markedly elevated *erb*B-3 mRNA levels were demonstrated in certain human tumor cell lines.

Accordingly, in a principal embodiment, the present invention relates to a DNA segment having a nucleotide sequence that encodes an *erb*B-3 gene or a unique portion thereof. This portion of an *erb*B-3 gene includes at least about 12 to 14 nucleotides which are sufficient to allow formation of a stable duplex with a DNA or RNA segment having sequences complementary to those in this portion of an *erb*B-3 gene. Further, this unique portion of an *erb*B-3 gene, of course, has a sequence not present in an *erb*B or an *erb*B-2 gene. In other words, the sequence of this portion of an *erb*B-3 gene differs in at least one nucleotide from the sequence of any other DNA segment. In one embodiment, this DNA segment is exemplified by a human genomic DNA fragment that is produced by cleavage with the *Sac*I restriction enzyme, has a size of about 90 kbp, and is detectable by nucleic acid hybridization with a probe derived from the v-*erb*B gene only under reduced stringency hybridization conditions, as described in Example 1. By application of the nucleic acid hybridization and cloning methods described in the present disclosure, without undue experimentation, one of ordinary skill in the art of recombinant DNA is enabled to identify and isolate DNA fragments related to the present human DNA fragment comprising a nucleotide sequence that encodes at least a portion of a mammalian *erb*B-3 gene other than the human *erb*B-3 gene. Application of the genomic DNA fragment of the *erb*B-3 gene as a probe in hybridization methods also enables one of ordinary skill in the art to obtain an entire *erb*B-3 gene, by sequential isolation of overlapping fragments adjoining the present fragment, i.e., by an approach known in the art as chromosome walking.

The present disclosure describes the partial nucleotide sequence of the human genomic 9 kbp *Sac*I DNA

fragment, within the region of homology of the v-*erb*B gene; however, the methods in the present disclosure further enable the isolation and determination of the sequence of the entire 9 kbp human genomic DNA fragment according to the present invention. Accordingly, the present invention further relates to a DNA segment having the nucleotide sequence, or a unique portion thereof, of a human genomic DNA fragment that is produced by cleavage with the *Sac*I restriction enzyme, has a size of about 9 kbp, and is detectable by nucleic acid hybridization with a probe derived from the v-*erb*B gene only under reduced stringency hybridization conditions, as described in Example 1. By extension of the chromosome walking approach noted above, the present invention further enables one of ordinary skill in the art to determination of the sequences of related DNA fragments comprising the complete human *erb*B-3 gene as well as *erb*B-3 genes of, for example, mammals other than human.

In the application of the present *Sac*I DNA fragment or any portion thereof as a probe for nucleic acid hybridization, the fragment is amplified, for example, by the *in vitro* polymerase chain reaction method (PCR; see U.S. Patent 4,683,202; U.S. Patent 4,683,195; and Saiki et al., 1985, *Science* 230:1350-54) or by standard methods of molecular cloning. For example, a clone of the human *erb*B-3 gene DNA segment according to the present invention is exemplified by a recombinant clone of a normal human thymus DNA fragment, herein designated as the E3-1 genomic clone, having the partial restriction enzyme map defined in Figure 2 and the partial DNA sequence defined in Figure 3 of the present application. Isolation and characterization of genomic clone E3-1 is described in Example 2, below.

Analysis of the nucleotide sequences of the human genomic DNA segment according to the present invention reveals that the nucleotide sequence encodes three open reading frames bordered by splice junction consensus sequences which define the boundaries between non-

translated intron sequences and the translated exons (Fig. 2). The predicted amino acid sequences of the three exons are highly similar to three regions which are contiguous in the tyrosine kinase domains of V-*erb*B, as well as human EGF-R and *erb*B-2 proteins. Moreover, the predicted amino acid sequences of this human genomic clone are included in a larger open reading frame in complementary DNA (cDNA) clones of an mRNA species that is detected by hybridization of a probe derived from the human genomic DNA clone.

Accordingly, the present invention also relates to a DNA segment having a nucleotide sequence of an *erb*B-3 gene in which that nucleotide sequence encodes the amino acid sequence of an *erb*B-3 gene or a unique portion thereof. In other words, the sequence of this portion of an *erb*B-3 amino acid sequence differs in at least one amino acid residue from the amino acid sequence encoded by any other DNA segment. This portion of an *erb*B-3 amino acid sequence includes at least about 4 to 6 amino acids which are sufficient to provide a binding site for an antibody specific for this portion of the *erb*B-3 polypeptide. Further, this unique portion of an *erb*B-3 amino acid sequence, of course, includes sequences not present in an *erb*B or an *erb*B-2 gene. In particular, the present invention relates to such a DNA segment for which this amino acid sequence or unique portion thereof is that of the polypeptide product of the human *erb*B-3 gene. This DNA segment is exemplified by the human genomic DNA clone E3-1, above, as well as by human cDNA closed designated E3-6, E3-8, E3-9, E3-11 and E3-16, which are described in Example 3 below. A preferred embodiment of this DNA segment that encodes the amino acid sequence of the entire polypeptide product of the human *erb*B-3 gene is human cDNA clone E3-16 having the nucleotide sequence defined in Figure 4 and having the predicted amino acid sequence defined in Figure 4.

The DNA segments according to this invention are useful for detection of expression of *erb*B-3 genes in normal and tumor tissues, as described in Example 5 below.

Therefore, in yet another aspect, the present invention relates to a bioassay for detecting *erb*B-3 mRNA in a biological sample comprising the steps of: i) contacting that biological sample with a DNA segment of this invention under conditions such that a DNA:RNA hybrid molecular containing this DNA segment and complementary RNA can be formed; and ii) determining the amount of that DNA segment present in the resulting hybrid molecule. Findings described in Example 5, below, indicate that increased *erb*B-3 expression, as detected by this method of this invention, plays a role in some human malignancies, as is the case for the EGF-R (*erb*B) and *erb*B-2 genes.

Of course, it will be understood by one skilled in the art of genetic engineering that in relation to production of *erb*B-3 polypeptide products, the present invention also includes DNA segments having DNA sequences other than those in the present examples that also encode the amino acid sequence of the polypeptide product of an *erb*B-3 gene. For example, it is known that by reference to the universal genetic code, standard genetic engineering methods can be used to produce synthetic DNA segments having various sequences that encode any given amino acid sequence. Such synthetic DNA segments encoding at least a portion of the amino acid sequence of the polypeptide product of the human *erb*B-3 gene also fall within the scope of the present invention. Further, it is known that different individuals may have slightly different DNA sequences for any given human gene and, in some cases, such mutant or variant genes encode polypeptide products having amino acid sequences which differ among individuals without affecting the essential function of the polypeptide product. Still further, it is also known that many amino acid substitutions can be made in a polypeptide product by genetic engineering methods without affecting the essential function of that polypeptide. Accordingly, the present invention further relates to a DNA segment having a nucleotide sequence that encodes an amino acid sequence differing in at least one amino acid from the

the present invention further relates to a DNA segment having a nucleotide sequence that encodes an amino acid sequence differing in at least one amino acid from the amino acid sequence of human *erb*B-3, or a unique portion thereof, and having greater overall similarity to the amino acid sequence of human *erb*B-3 than to that of any other polypeptide. The amino acid sequence of this DNA segment includes at least about 4 to 6 amino acids which are sufficient to provide a binding site for an antibody specific for the portion of a polypeptide containing this sequence. In a preferred embodiment, this DNA segment encodes an amino acid sequence having substantially the function of the human *erb*B-3 polypeptide. As noted above, the predicted *erb*B-3 polypeptide is a 148 Kd transmembrane polypeptide with structural features identifying it as a member of the *erb*B receptor family.

The similarity of the amino acid sequence of the present invention with that of an *erb*B-3 amino acid sequence is determined by the method of analysis defined by the sequence alignment and comparison algorithms described by Pearson and Lipman (Pearson, W.R. & Lipman, D. J., 1988, *Proc. Nat. Acad. Sci. U.S.A. 85*:2444-48). This comparison contemplates not only precise homology of amino acid sequences, but also substitutions of one residue for another which are known to occur frequently in families of evolutionarily related proteins sharing a conserved function.

The present invention further relates to a recombinant DNA molecule comprising DNA segment of this invention and a vector. In yet another aspect, the present invention relates to culture of cells transformed with a DNA segment according to this invention. These host cells transformed with DNAs of the invention include both higher eukaryotes, including animal, plant and insect cells, and lower eukaryotes, such as yeast cells, as well as prokaryotic hosts including bacterial cells such as those of *E. coli* and *Bacillus subtilis*. These aspects of the inven-

transformed with a DNA of the invention, wherein the transforming DNA is capable of being expressed to produce the functional polypeptide of an *erb*B-3 gene. For example, mammalian cells (COS-1) transformed with the pSV2 gpt vector carrying the E3-16 cDNA, are prepared according to well-known methods, such as those described in U.S. Patent Application 07/308,302 of Matsui et al., filed February 9, 1989; see also Pierce, J. H. et al., 1988, *Science* 239:628-631; and Matsui, T., Heidaran, M., Miki, T., Popescu, N., La Rochelle, W., Kraus, M., Pierce, J. & Aaronson, S., 1989, *Science* 243:800-804). Briefly, cDNA expression plasmids are constructed by introducing the *erb*B-3-related cDNA encompassing all the nucleotides in the open reading frame into the pSV2 gpt vector into which the simian sarcoma virus long-terminal-repeat (LTR) had been engineered as the promoter, as previously described in detail. Transient expression an *erb*B-3 gene in such recombinant vectors is achieved by transection into COS-1 cells.

Stable expression of an *erb*B-3 gene can also be obtained with mammalian expression vectors such as the pZIPNEOSVX vector (Cepko, C. L., Roberts, B.E. and Mulligan, R. C., 1984, *Cell* 37:1053-62). For example, a eukaryotic expression vector was engineered by cloning the full-length *erb*B-3 coding sequence derived from cDNA clone E3-16 into the BamHI site of the pZIPNEOSVX vector DNA adapting the DNA fragments with synthetic oligonucleotides. NIH3T3 cells were transfected with 1 μg of recombinant expression vector DNA (LTR*erb*B-3) and selected with the resistance marker antibiotic G418. To detect expression of *erb*B-3, a polyclonal rabbit antiserum was raised against a synthetic peptide (amino acid positions 1191-1205) within the predicted carboxyl terminus of the *erb*B-3 coding sequence. As shown in Figure 8, immunoblotting analysis led to detection of the *erb*B-3 protein (Fig. 8A). The specificity of *erb*B-3 protein detection was demonstrated by preincubating the antiserum with the homologous peptide (Fig. 8B). Moreover, the normal 180 kD

*erb*B-3 protein was specifically detected with the polyclonal antiserum only in cells transfected with the recombinant *erb*B-3 expression vector, while control NIH3T3 cells that were not transfected with the vector were negative. The stably transfected NIH3T3 cells are useful as *erb*B-3 receptor protein sources for testing potential candidates for an *erb*B-3-specific ligand, analysis of the biological activity, as well as generation of monoclonal antibodies raised against the native *erb*B-3 protein. An *erb*B-3-specific liquid is identified by detection of autophosphorylation of the *erb*B-3 receptor protein, stimulation of DNA synthesis or induction of the transformed phenotype of the LTR*erb*B-3 transfected NIH3T3 cells.

Alternatively, other transformed cell systems are available for functional expression of receptors of the *erb*B receptor family, for example, a system based on the 32D cell line, a mouse hematopoietic cell line normally dependent on interleukin-3 (Il-3) for survival and proliferation. Recent studies have established that introduction of an expression vector for the EGF-R in these cells leads to effective coupling with EGF mitogenic signal transduction pathways, thereby allowing a ligand of the EGF-R to replace Il-3 in supporting survival and growth of the 32D cells. By employing the known methods described for the EGF-R, for example (Pierce, J. H. et al., 1988, *supra*), the E3-16 cDNA of the present invention is expressed to produce functional receptors in 32D cells which are then useful for examining the biological function of these *erb*B-3 receptors, for instance, the specificity of their ligand binding capacity and coupling capacities to secondary messenger systems. Thus, by so using gene expression methods described herein with the DNAs of the present invention, especially the preferred E3-16 cDNA clone, one of ordinary skill in the art, without undue experimentation, can construct cell systems which fall within the scope of this invention, for determining the mechanisms of *erb*B-3 regulatory processes. Accordingly,

the present invention also relates to a bioassay for testing potential analogs of ligands of *erb*B-3 receptors for the ability to affect an activity mediated by *erb*B-3 receptors, comprising the steps of: i) contacting a molecule suspected of being a ligand with *erb*B-3 receptors produced by a cell producing functional *erb*B-3 receptors; and ii) determining the amount of a biological activity mediated by those *erb*B-3 receptors.

Various standard recombinant systems, such as those cited above as well as others known in the art, are suitable as well for production of large amounts of the novel *erb*B-3 receptor protein using methods of isolation for receptor proteins that are well known in the art. Therefore, the present invention also encompasses an isolated polypeptide having at least a portion of the amino acid sequence defined in Figure 4.

This invention further comprises an antibody specific for a unique portion of the human *erb*B-3 polypeptide having the amino acid sequence defined in Figure 4, or a unique portion thereof. In this embodiment of the invention, the antibodies are monoclonal or polyclonal in origin, and are generated using *erb*B-3 receptor-related polypeptides or peptides from natural, recombinant or synthetic chemistry sources. These antibodies specifically bind to an *erb*B-3 protein which includes the sequences of such polypeptide. In other words, these antibodies bind only to *erb*B-3 receptor proteins and not to *erb*B (EGF-R) or *erb*B-2 proteins. Also, preferred antibodies of this invention bind to an *erb*B-3 protein when that protein is in its native (biologically active) conformation.

Fragments of antibodies of this invention, such as Fab or F(ab)′ fragments, which retain antigen binding activity and can be prepared by methods well known in the art, also fall within the scope of the present invention. Further, this invention comprises a pharmaceutical composition of the antibodies of this invention, or an active fragment thereof, which can be prepared using materials and methods for preparing pharmaceutical compositions for

administration of polypeptides that are well known in the art and can be adapted readily for administration of the present antibodies without undue experimentation.

These antibodies and active fragments thereof, can be used, for example, for specific detection or purification of the novel *erb*B-3 receptor. Such antibodies could also be used in various methods known in the art for targeting drugs to tissues with high levels of *erb*B-3 receptors, for example, in the treatment of appropriate tumors with conjugates of such antibodies and cell killing agents. Accordingly, the present invention further relates to a method for targeting a therapeutic drug to cells having high levels of *erb*B-3 receptors, comprising the steps of i) conjugating an antibody specific for an *erb*B-3 receptor, or an active fragment of that antibody, to the therapeutic drug; and ii) administering the resulting conjugate to an individual with cells having high levels of *erb*B-3 receptors in an effective amount and by an effective route such that the antibody is able to bind to the *erb*B-3 receptors on those cells.

The antibody of this invention is exemplified by rabbit antisera containing antibodies which specifically bind to *erb*B-3 protein. Such receptor specific antisera are raised to synthetic peptides representing a unique portion of the *erb*B-3 amino acid sequence, having six or more amino acids in sequences which are sufficient to provide a binding site for an antibody specific for this portion of the *erb*B-3 polypeptide. Further, this unique portion of an *erb*B-3 amino acid sequence, of course, includes sequences not present in an *erb*B or an *erb*B-2 amino acid sequence, as predicted by the respective cDNA sequences. The *erb*B-3 specific anti-peptide antibody of the present invention is exemplified by an anti-peptide antibody in polyclonal rabbit antiserum raised against the synthetic peptide having the sequence (in single letter amino acid code) EDEDEEYEYMNRRRR representing amino acid positions 1191-1205 in the predicted sequence of the *erb*B-3 polypeptide. The specific detection of *erb*B-3 polypep-

tide with this antiserum is illustrated in mammalian cells transformed with an expression vector carrying a human *erb*B-3 cDNA (see Figures 8A and 8B).

Antibodies to peptides are prepared by chemically synthesizing the peptides, conjugating them to a carrier protein, and injecting the conjugated peptides into rabbits with complete Freund's adjuvant, according to standard methods of peptide immunization. For example, the peptide is synthesized by standard methods (Merrifield, R. B., 1963, *J. Amer. Soc.*, 85:2149) on a solid phase synthesizer. The crude peptide is purified by HPLC and conjugated to the carrier, keyhole limpet hemocyanin or bovine thyroglobulin, for example, by coupling the amino terminal cysteine to the carrier through a maleimido linkage according to well known methods (e.g., Lerner, R. A. et al., 1981, *Proc. Nat. Acad. Sci. USA*, 78:3403). In one standard method of peptide immunology, rabbits are immunized with 100 µg of the *erb*B-3 peptide-carrier conjugate (1 mg/ml) in an equal volume of complete Freund's adjuvant and then boosted at 10-14 day intervals with 100 µg of conjugated peptide in incomplete Freund's adjuvant. Additional boosts with similar doses at 10-14 day intervals are continued until anti-peptide antibody titer, as determined, for example, by routine ELISA assays, reaches a plateau.

Thus, by following the teachings of the present disclosure, including application of generally known immunological methods cited herein, one of ordinary skill in the art is able to obtain *erb*B-3-specific antibodies and use them in a variety of immunological assays, for example, for diagnostic detection of unusually high or low expression in normal or tumor tissues. Thus, the present invention also relates to a bioassay for detecting an *erb*B-3 antigen in a biological sample comprising the steps of: i) contacting that sample with an antibody of the present invention specific for an *erb*B-3 polypeptide, under conditions such that a specific complex of that antibody and that antigen can be formed; and ii) determin-

ing the amount of that antibody present in the form of those complexes.

* * * *

The present invention may be understood more readily by reference to the following detailed description of specific embodiments and the Examples and Figures included therein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show detection of v-*erb*B-related DNA fragments in DNAs from normal human thymus (lane 1), human mammary tumor lines MDA-MB468 (lane 2), and SK-BR-3 (lane 3). Hybridization was conducted at reduced (Fig. 2A) or intermediate (Fig. 2B) stringency conditions. The arrow denotes a novel 9 kilobase pair (kbp) *erb*B-related restriction fragment distinct from those of the EGF-R gene (*erb*B) and *erb*B-2.

Figure 2 shows genomic and cDNA cloning of *erb*B-3. The region of v-*erb*B homology within the genomic 9 kbp *Sac*I insert of λE3-1 was subcloned into the plasmid pUC (pE3-1) and subjected to nucleotide sequence analysis. The three predicted exons are depicted as solid boxes. *erb*B-3 cDNA clones were isolated from oligo dT-primed libraries of mRNAs from normal human placenta (shaded bars) and the breast tumor cell line MCF-7 (open bar). The entire nucleotide sequence was determined for both strands on *erb*B-3 complementary DNA from normal human placenta and upstream of the 5′ *Xho*I site on pE3-16. The coding sequence is shown as a solid bar and splice junctions of the three characterized genomic exons are indicated by vertical white lines. Solid lines in the cDNA map represent untranslated sequences. Restriction sites: A=*Acc*I, Av=*Ava*I, B=*Bam*HI, Bg=*Bg*III, E=*Eco*RI, H=*Hind*III, K=*Kpn*I, M=*Mst*II, P=*Pst*I, S=*Sac*I, Sm=*SmaI*, Sp=*Spe*I.

Figure 3 shows the nucleotide sequence of the region of v-*erb*B homology in the human *erb*B-3 gene derived from human genomic DNA clone E3-1, in the 1.5 kbp region from the *Eco*RI to the *Pst*I sites. This region contains three open reading frames bordered by splice junction

consensus sequences (underlined). The predicted amino acid sequences of the three exons are shown in three letter code above the relevant DNA sequences.

Figure 4 shows the nucleotide sequence of the cDNA encoding the *erb*B-3 polypeptide and the predicted amino acid sequence of that polypeptide.

Figure 5 shows comparison of the predicted amino acid sequence of the *erb*B-3 polypeptide with other receptor-like tyrosine kinases. The amino acid sequence is shown in single letter code and is numbered on the left. The putative extracellular domain (light shading) extends between the predicted signal sequence (solid box) at the amino-terminus and a single hydrophobic transmembrane region (solid box) within the polypeptide. The two cysteine clusters (Cys) in the extracellular domain and the predicted tyrosine kinase domain (TK) within the cytoplasmic portion of the polypeptides are outlined by dark shading. The putative ATP-binding site at the amino-terminus of the TK domain is circled. Potential auto-phosphorylation sites within the carboxyl-terminal domain (COOH) are indicated by asterisks. Potential N-linked glycosylation sites (•—) are marked above the amino acid sequence. The percentage of amino acid homology of *erb*B-3 in individual domains with *erb*B-2, EGF-R, *met*, *eph*, insulin receptor (IR), and *fms* is listed below. Less than 16% identity is denoted by (-).

Figure 6 shows the assignment of the genomic locus of *erb*B-3 was assigned to human chromosomal locus 12q13. A total of 142 grains were localized on the 400-band ideogram. As depicted in the diagram, specific labeling of chromosome 12 was observed, where 38 out of 51 grains were localized to band q13.

Figures 7A and 7B show the elevated *erb*B-3 transcript levels in human mammary tumor cell lines. A Northern blot containing 10 μg total cellular RNA from AB589 mammary epithelial cells (lane 1), as well as mammary tumor cell lines MDA-MB415 (lane 2) and MDA-MB453 (lane 3) was hybridized with an *erb*B-3 cDNA probe (Fig.

7A). Following signal decay the same blot was rehybridized with a human β-actin cDNA probe (Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. & Kedes, L., 1983, *Mol. Cell Biol.* 3:787-795).

Figures 8A and 8B show the expression of a human *erb*B-3 polypeptide in cells transformed by a cDNA segment as detected by an *erb*B-3-specific antipeptide antiserum. Cellular lysates (100 μg of each sample) were electrophoresed and transferred to nitrocellulose membranes for analysis by Western blotting. Figure 8A shows the detection of *erb*B-3 polypeptide with the antiserum. Figure 8B shows the preincubation of the antiserum with homologous peptide. Antibody blocking indicates binding specificity. Lane 1: Selected cultures of NIH3T3 cells transfected with 1 μg LTR*erb*B-3 expression vector. Lane 2: control NIH3T3 cells.

DESCRIPTION OF SPECIFIC EMBODIMENTS

The identification of a third member of the *erb*BEGF receptor family of membrane spanning tyrosine kinases and the cloning of its full length coding sequence is described in the Examples herein. The presence of apparent structural domains resembling those of the EGF receptor suggests the existence of an extracellular binding site for a ligand. The structural relatedness of the extracellular domain of the *erb*B-3 receptor with that of the EGF receptor indicates that one or more of an increasing number of EGF-like ligands (Shoyab, M., Plowman, G. D., McDonald, V. L., Bradley, J. G. & Todaro, G. J., 1989, *Science* 243:1074-1076) interacts with the *erb*B-3 product. Accordingly, the *erb*B-3 gene is expected to play important roles in both normal and neoplastic processes, as is known for the EGF-R and *erb*B-2 genes.

Despite extensive collinear homology with the EGF receptor and *erb*B-2, distinct regions within the predicted *erb*B-2, coding sequence revealed relatively higher degrees of divergence. For example, its carboxyl terminal domain failed to exhibit significant collinear identity scores with either *erb*B-2 or EGF-R. The divergence at the

carboxyl terminus also accounts for minor size differences among the three polypeptides of *erb*B-3, *erb*B-2, and EGF-R, which possess estimated molecular weights of 148 kilodaltons (kd), 138 kd, and 131 kd, respectively. Within the tyrosine kinase domain, which represents the most conserved region of the predicted *erb*B-3 protein, a short stretch of 29 amino acids closer to the carboxyl terminus than the ATP binding site differed from regions of the predicted *erb*B-2 and EGF-R coding sequence in 28 and 25 positions, respectively. Such regions of higher divergence in their cytoplasmic domains are likely to confer different functional specificity to these closely related receptor-like molecules. Thus, mutations or other alterations in expression of the *erb*B-3 gene are likely to cause cancers or genetic disorders different from those associated with such defects in the *erb*B and *erb*B-2 genes.

Chromosomal mapping localized *erb*B-3 to human chromosome 12 at the q11-13 locus, whereas the related EGF-R and *erb*B-2 genes are located at chromosomal sites 7p12-13 and 17p12-21.3, respectively. Thus, each gene appears to be localized to a region containing a different homeobox and a different collagen chain gene locus. Keratin type I and type II genes also map to regions of 12 and 17, respectively, consistent with the different localizations of *erb*B-3 and *erb*B-2, respectively. Thus, the DNA segments of the present invention represent novel probes to aid in genetic mapping of any heritable diseases which are associated with chromosomal aberrations in the vicinity of the 12q11-13 locus.

There is evidence for autocrine as well as paracrine effectors of normal cell proliferation. The former are factors that are produced by the same cells upon which they stimulate cell proliferation, whereas the latter factors are secreted by cells other than those that are affected by those factors. However, the inherent transforming potential of autocrine growth factors suggests that growth factors most commonly act on their target cell populations by a paracrine route. The present survey of

*erb*B-3 gene expression indicates its normal expression in cells of epithelial and neuroectodermal derivation. Comparative analysis of the three *erb*B receptor-like genes in different cell types of epidermal tissue revealed that keratinocytes expressed all three genes. In contrast, melanocytes and stromal fibroblasts specifically lacked EGF-R and *erb*B-3 transcripts, respectively. Thus, melanocytes and stromal fibroblasts may be sources of paracrine growth factors for EGF-R and *erb*B-3 products, respectively, that are expressed by the other cell types residing in close proximity in epidermal tissues.

Given that both *erb*B and *erb*B-2 have been casually implicated in human malignancy, the present findings (Example 5) that the *erb*B-3 transcript is overexpressed in a significant fraction of human mammary tumor cell lines indicates that this new member of the EGF-R receptor family also plays an important role in some human malignancies.

<u>Example 1. Identification of a human DNA fragment related to the *erb*B proto-oncogene family</u>. In an effort to detect novel *erb*B-related genes, human genomic DNA was cleaved with a variety of restriction endonucleases and subjected to Southern blot analysis with v-*erb*B as a probe. Normal mammary epithelial cells AB589 (Walen, K. H. & Stampfer, M. R., 1989, *Cancer. Genet. Cytogenet.* 37:249-261) and immortalized keratinocytes RHEK have been described previously (Rhim, J. S., Jay, G., Arnstein, P., Price, F. M., Sanford, K. K. & Aaronson, S. A., 1985, *Science* 227:1250-52). Normal human epidermal melanocytes (NHEM) and keratinocytes (NHEK) were obtained from Clonetics. Sources for human embryo fibroblasts (Rubin, J. S., Osada, H., Finch, P. W., Taylor, W. G., Rudikoff, S., & Aaronson, S. A., 1989, *Proc. Nat. Acad. Sci. USA* 86:802-806) or mammary tumor cell lines SK-BR-3, MDA-MB468, MDA-MB453, and MDA-MB415 (Kraus, M. H., Popescu, N. C., Amsbaugh, S. C. & King, C. R., 1987 *EMBO. J.* 6:605-610) have been described. For nucleic acid RNA hybridization, DNA and RNA were transferred to nitrocellulose

membranes as previously described (Kraus, K. H., et al., 1987, *supra*). High stringency hybridization was conducted in 50% formamide and 5xSSC at 42°C. Filters were washed at 50°C in 0.1xSSC. Reduced stringency hybridization of DNA was carried out in 30% formamide followed by washes in 0.6xSSC, while intermediate stringency was achieved by hybridization in 40% formamide and washing in 0.25xSSC. For the specific results depicted in Fig. 1, DNAs were restricted with *Sac*I and hybridized with probe specific for an oncogenic viral form of the *erb*B gene, v-*erb*B, spanning from the upstream BamHI site to the *Eco*RI site in the avian erythroblastosis proviral DNA (Vennstrom, B., Franshier, L., Moscovici, C. & Bishop, J. M., 1980, *J. Virol.* 36:575-585).

Under reduced stringency hybridization, four *Sac*I restriction fragments were detected. Two were identified as EGF-R gene fragments by their amplifications in the mammary tumor cell line MDA-MB468 (Fig. 1A, lane 1,2) known to contain EGF-R gene amplification and one as an *erb*B-2 specific gene fragment due to its increased signal intensity in another mammary tumor cell line, SK-BR-3, known to have *erb*B-2 amplified (Fig. 1A, lane 1,3). However, a single 9 kbp *Sac*I fragment exhibited equal signal intensities in DNAs from normal human thymus, SK-BR-3 and a line with high levels of EGF-R, A431 (Fig. 1A). When the hybridization stringency was raised by 7°C, this fragment did not hybridize, whereas EGF-R and *erb*B-2 specific restriction fragments were still detected with v-*erb*B as a probe (Fig. 1B). Taken together, these findings suggested the specific detection of a novel v-*erb*B-related DNA sequence within the 9 kbp *Sac*I fragment.

<u>Example 2. Cloning of the human DNA fragment related to *erb*B</u>. For further characterization a normal human genomic library was prepared from *Sac*I cleaved thymus DNA enriched for 8 to 12 kbp fragments. For convenience, bacteriophage λsep6-lac5 was obtained from L. Prestidge and D. Hogness (Stanford University); many other cloning vectors derived from phage λ or other genomes can

be used for cloning this DNA fragment according to standard recombinant DNA methods that are well known in the art. Purified phage DNA was subjected to *cos*-end ligation, restriction with *Sac*I, and fractionation in a continuous 10-40% sucrose gradient. A genomic library was prepared by ligating *Sac*I restriction fragments of normal human thymus DNA in the molecular weight range of 8 kbp to 12 kbp (isolated by sucrose gradient sedimentation) with the purified phage arms. Ten recombinant clones detected by v-*erb*B under reduced stringency conditions did not hybridize with human EGF-R or *erb*B-2 cDNA probes at high stringency. As shown in the restriction map of a representative clone with a 9 kbp insert, the region of v-*erb*B homology was localized by hybridization analysis to a 1.5 kbp segment spanning from the *Eco*RI to the downstream *Pst*I site.

The nucleotide sequence of a portion of a clone of the novel human genomic DNA fragment related to *erb*B was determined for both DNA strands by the dideoxy chain termination method (Sanger, F., Nicklen, S. & Coulson, A. R., 1977, *Proc. Nat. Acad. Sci. USA*. 74:5463-67) using supercoiled plasmid DNA as template. The nucleotide sequence was assembled and translated using IntelliGenetics software. Amino acid sequence comparison was performed with the alignment program by Pearson and Lipman (Pearson, W. R. & Lipman, D. J., 1988, *supra*) as implemented on the computers of the NCI Advanced Scientific Computing Laboratory. Hydrophobic and hydrophilic regions in the predicted protein were identified according to Kyte and Doolittle (Kyte, J. & Doolittle, R. F., 1982, *J. Mol. Biol*. 157:105-132). Nucleotide sequence analysis revealed that the region of v-*erb*B homology in the 1.5 kbp segment from the *Eco*RI to the *Pst*I contained three open reading frames bordered by splice junction consensus sequences (Fig. 2). Computerized comparisons of the predicted amino acid sequence of these three open reading frames with other known proteins revealed the highest identity scores of 64% to 67% to three regions which are

contiguous in the tyrosine kinase domains of v-*erb*B, as well as human EGF-R and *erb*B-2 proteins. Furthermore, all splice junctions of the three characterized exons in the new gene were conserved with *erb*B-2. Amino acid sequence homology to other known tyrosine kinases was significantly lower, ranging from 39% to 46%.

A single 6.2 kb specific mRNA was identified by Northern blot analysis of human epithelial cells using the 150 bp *Spe*I-*Acc*I exon-containing fragment as probe (Fig. 2). Under the stringent hybridization conditions employed, this probe detected neither the 5 kb *erb*B-2 mRNA nor the 6 kb and 10 kb EGF-R mRNAs. All of these findings suggested that the present work has identified a new functional member of the *erb*B proto-oncogene family, which tentatively has been designated as *erb*B-3.

<u>Example 3. Cloning and characterization of cDNAs for the mRNA of the human *erb*B-3 gene</u>. In an effort to characterize the entire *erb*B-3 coding sequence, overlapping cDNA clones were isolated from oligo dT-primed cDNA libraries from sources with known *erb*B-3 expression, utilizing gene-specific genomic exons or cDNA fragments as probes. In brief, an oligo dT-primed human placenta cDNA library in λgt11 was obtained from Clontech. MCF-7 cDNA was prepared by first strand synthesis from 5 μg poly A^+ RNA using an oligo dT containing linker-primer and Mo-MuLV reverse transcriptase, followed by second strand synthesis with DNA polymerase I, RNaseH, and subsequent T4 DNA polymerase treatment. Double-stranded cDNA was directionally cloned into the *Sfi*I site of λpCEV9 using specific linker adapter oligonucleotides (Miki, T., Matsui, T., Heidaran, M. A. & Aaronson, S. A., 1989, *Gene* 83:137-146; see also, U.S. Application Ser. No. 07/386,053 of Miki et al., filed July 28, 1989). Following plaque purification, phage DNA inserts were subcloned into pUC-based plasmid vectors for further characterization. The clones were initially characterized by restriction analysis and hybridization to the mRNA, and were subsequently subjected to nucleotide sequence analysis. Clones designated pE3-6,

pE3-8, pE3-9, and pE3-11 carrying inserts with molecular weights ranging from 1.3 kpb to 4.3 kbp were isolated from a human placenta library, whereas the pE3-16 clone containing a 5 kbp insert was obtained by screening the MCF-7 cDNA library with the upstream most coding sequence of pE3-11 as a probe. The clones pE3-8, pE3-9, pE3-11, and pE3-16 contained identical 3′ ends terminating in a poly A stretch (Fig. 2).

The complete coding sequence of *erb*B-3 was contained within a single long open reading frame of 4080 nucleotides extending from position 46 to an in-frame termination codon at position 4126. The most upstream ATG codon at position 100 was the likely initiation codon, as it was preceded by an in-frame stop codon at nucleotide position 43 and fulfilled the criteria of Kozak for an authentic initiation codon. The open reading frame comprised 1342 codons predicting a 148 kd polypeptide. Downstream from the termination codon, multiple stop codons were present in all frames. As shown in Fig. 5, the deduced amino acid sequence of the *erb*B-3 polypeptide predicted a transmembrane receptor tyrosine kinase most closely related to EGF-R and *erb*B-2. A hydrophobic signal sequence of *erb*B-3 was predicted to comprise the 19 amino-terminal amino acid residues. Cleavage of this signal sequence between glycine at position 19 and serine at position 20 would generate a processed polypeptide of 1323 amino acids with an estimated molecular weight of 145 kd. A single hydrophobic membrane spanning domain encompassing 21 amino acids was identified within the coding sequence separating an extracellular domain of 624 amino acids from a cytoplasmic domain comprising 678 amino acids (Fig. 5).

The putative *erb*B-3 ligand-binding domain was 43% and 45% identical in amino acid residues with the predicted *erb*B-2 and EGF-R protein, respectively. Within the extracellular domain, all 50 cysteine residues of the processed *erb*B-3 polypeptide were conserved and similarly spaced when compared to the EGF-R and *erb*B-2. Forty-seven cysteine residues were organized in two clusters contain-

ing 22 and 25 cysteines respectively, a structural hallmark of this tyrosine kinase receptor subfamily (see, for example, Yamamoto, T., Ikawa, S., Akiyama, T., Semba, K., Nomura, N., Miyajima, N., Saito, T. & Toyoshima, K., 1986, *Nature* 319:230-234). Ten potential N-linked glycosylation sites were localized within the *erb*B-3 extracellular domain. In comparison with the EGF-R and *erb*B-2 proteins, five and two of these glycosylation sites were conserved, respectively. Among these, the site proximal to the transmembrane domain was conserved among all three proteins (Fig. 5).

Within the cytoplasmic domain, a core of 277 amino acids from position 702 through 978 revealed the most extensive homology with the tyrosine kinase domains of EGF-R and *erb*B-2. In this region 60% or 62% of amino acid residues were identical and 90% or 89% were conserved, respectively. This stretch of amino acid homology coincides with the minimal catalytic domain of tyrosine kinases (Hanks, S. K., Quinn, A. M. & Hunter, T., 1988, *Science* 241:42-52). There was significantly lower homology with other tyrosine kinases (Fig. 5). The consensus sequence for an ATP-binding site GxGxxG (Hanks, S. K. et al., 1988, *supra*) was identified at amino acid positions 716 through 721. This sequence as well as a lysine residue located 21 amino acid residues further toward the carboxyl terminus were conserved between the three *erb*B-related receptors. Taken together these findings defined the region between amino acid position 702 and 978 as the putative catalytic domain of the *erb*B-3 protein (Fig. 5).

The most divergent region of *erb*B-3 compared to either EGF-R or *erb*B-2 was its carboxyl terminus comprising 364 amino acids. This region showed a high degree of hydrophilicity and the frequent occurrence of proline and tyrosine residues. Among these tyrosine residues, those at positions 1197, 1199, and 1262 matched closest with the consensus sequence for putative phosphorylation sites. The peptide sequence YEYMN, encompassing tyrosine 1197 and 1199, was repeated at positions 1260-1264 and was at both

locations surrounded by charged residues, providing an environment of high local hydrophilicity. These observations render tyrosines 1197, 1199 and 1262 likely candidates for autophosphorylation sites of the *erb*B3 protein.

Example 4. Chromosomal mapping of the human *erb*B-3 gene. The chromosomal location of the *erb*B-3 gene was determined by *in situ* hybridization (Popescu, N. C., King, C. R. & Kraus, M. H., 1989, *Genomics* 4:362-366) with a ^{3}H-labeled plasmid containing the amino-terminal *erb*B-3 coding sequence. A total of 110 human chromosome spreads were examined prior and subsequent to G banding for identification of individual chromosomes. A total of 142 grains were localized on a 400-band ideogram. Specific labeling of chromosome 12 was observed, where 38 out of 51 grains were localized to band q13 (Fig. 6). Thus, the genomic locus of *erb*B-3 was assigned to 12q13. In this region of chromosome 12, several genes have previously been mapped including the melanoma-associated antigen ME491, histone genes and the gene for lactalbumin. In addition, two proto-oncogenes, *int*-1 and *gli* are located in close proximity to *erb*B-3.

Example 5. ErbB-3 expression in normal and malignant human cells. To investigate its pattern of expression, a number of human tissues were surveyed for the *erb*B-3 transcript. The 6.2 kb *erb*B-3 specific mRNA was observed in term placenta, postnatal skin, stomach, lung, kidney, and brain, while it was not detectable in skin fibroblasts, skeletal muscle or lymphoid cells. Among the fetal tissues analyzed, the *erb*B-3 transcript was expressed in liver, kidney, and brain, but not in fetal heart or embryonic lung fibroblasts. These observations indicate the preferential expression of *erb*B-3 in epithelial tissues and brain.

*Erb*B-3 expression was also investigated in individual cell populations derived from normal human epithelial tissues including keratinocytes, glandular epithelial cells, melanocytes, and fibroblasts. For comparison

levels of EGF-R and *erb*B-2 transcripts were analyzed. As shown in Table 1, *erb*B-3 mRNA levels were relatively high in keratinocytes, comparable with those of *erb*B-2 and EGF-R in these cells. Lower, but similar expression levels of each transcript were detected in cells derived from glandular epithelium. These findings are consistent with growth regulatory roles of all three receptor-like molecules in squamous and glandular epithelium. Whereas *erb*B-2 and EGF-R transcripts were also readily observed in normal fibroblasts, the same cells lacked detectable *erb*B-3 mRNA. In contrast, normal human melanocytes, which expressed both *erb*B-3 and *erb*B-2 at levels comparable with human keratinocytes, lacked detectable EGF-R transcripts. Thus, the expression patterns of these receptor-like molecules were different in specialized cell populations derived from epidermal tissues.

Table 1: Normal expression pattern of human *erb*B gene family members.

Cell Source of Transcripts	Gene	Relative RNA levels
Embryonic fibroblast (M426)	*erb*B-3	-
	*erb*B-2	+
	EGF-R	+
Skin fibroblast (501T)	*erb*B-3	-
	*erb*B-2	+
	EGF-R	+
Immortal keratinocyte (RHEK)	*erb*B-3	++
	*erb*B-2	++
	EGF-R	++
Primary keratinocyte (NHEK)	*erb*B-3	+
	*erb*B-2	+
	EGF-R	++
Glandular epithelium (AB589)	*erb*B-3	(+)
	*erb*B-2	(+)
	EGF-R	(+)
Melanocyte (NHEM)	*erb*B-3	++
	*erb*B-2	++
	EGF-R	-

Replicate Northern blots were hybridized with equal amounts (in cpm) of probes of similar specific activities for *erb*B-3, *erb*B-2, and EGF-R, respectively. Relative signal intensities were estimated: - not detectable, (+) weakly positive, + positive, ++ strongly positive.

To search for evidence of *erb*B-3 involvement in the neoplastic process, *erb*B-3 mRNA levels in a series of human tumor cell lines were surveyed. The *erb*B-3 transcript was detected in 36 of 38 carcinomas and 2 of 12 sarcomas while 7 tumor cell lines of hematopoietic origin lacked measurable *erb*B-3 mRNA. Markedly elevated levels of a normal-sized transcript were observed in 6 out of 17 tumor cell lines derived from human mammary carcinomas. By Southern blot analysis, neither gross gene rearrangement nor amplification was detected in the cell lines. Figure 7A shows the results of Northern blot analysis with control AB589 nonmalignant human mammary epithelial cells (lane 1) and two representative human mammary tumor lines, MDA-MB415 (lane 2) and MDA-MB453 (lane 3). Hybridization of the same filter with a human β-actin probe (Fig. 7B) verified actual levels of mRNA in each lane. Densitometric scanning indicated that the *erb*B-3 transcript in each tumor cell line was elevated more than 100 fold above that of the control cell line. Thus, overexpression of this new member of the *erb*B family, as in the case of the EGF-R and *erb*B-2 genes, is likely to play an important role in some human malignancies.

* * * *

For purposes of completing the background description and present disclosure, each of the published articles, patents and patent applications heretofore identified in this specification are hereby incorporated by reference into the specification.

The foregoing invention has been described in some detail for purposes of clarity and understanding. It will

also be obvious that various changes and combinations in form and detail can be made without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A DNA segment having a nucleotide sequence that encodes an *erb*B-3 gene or a unique portion thereof.

2. The DNA segment according to claim 1, wherein said gene is a mammalian *erbB*-3 gene.

3. The DNA segment according to claim 2, wherein said mammalian gene is a human *erbB*-3 gene.

4. A DNA segment having the nucleotide sequence, or a unique portion thereof, of a genomic DNA fragment that is produced by cleavage with the *Sac*I restriction enzyme, has a size of about 9 kbp, and is detectable by nucleic acid hybridization with a probe derived from the v-*erbB* gene only under reduced stringency hybridization conditions.

5. The DNA segment according to claim 4, wherein said segment is the human genomic DNA clone E3-1, or a unique portion thereof, said clone having the partial restriction enzyme map defined in Figure 2 and the partial DNA sequence defined in Figure 3.

6. The DNA segment according to claim 1, wherein said nucleotide sequence encodes the amino acid sequence of an *erbB*-3 gene or a unique portion thereof.

7. The DNA segment according to claim 6, wherein said amino acid sequence is that defined in Figure 4.

8. The DNA segment according to claim 7, comprising human cDNA clone E3-16 having the nucleotide sequence defined in Figure 4.

9. A DNA segment having a nucleotide sequence that encodes an amino acid sequence differing in at least one amino acid from the amino acid sequence of human *erbB*-3, or a unique portion thereof, and having greater overall similarity to the amino acid sequence of human *erbB*-3 than to that of any other polypeptide.

10. The DNA segment according to claim 9 that encodes an amino acid sequence having substantially the function of the human *erbB*-3 polypeptide.

11. An isolated polypeptide having an amino acid sequence encoded by the DNA segment according to claim 9.

12. A recombinant DNA molecule comprising the DNA segment to claim 1 and a vector.

13. A culture of cells transformed with the DNA segment according to claim 1.

14. An isolate polypeptide having the amino acid sequence defined in Figure 4, or a unique portion thereof.

15. A bioassay for detecting *erbB*-3 mRNA in a biological sample comprising the steps of:

i) contacting said biological sample with a DNA segment according to claim 1 under conditions such that a DNA:RNA hybrid molecule containing said DNA segment and complementary RNA can be formed; and

ii) determining the amount of said DNA segment present in said hybrid molecule.

16. A bioassay for testing potential analogs of ligands of *erbB*-3 receptors for the ability to affect an activity mediated by said *erbB*-3 receptors, comprising the steps of:

i) contacting a molecule suspected of being a ligand with *erbB*-3 receptors produced by a cell according to claim 11; and

ii) determining the amount of a biological activity mediated by said *erbB*-3 receptors in said cells.

17. An antibody specific for a unique portion of the polypeptide according to claim 14.

18. A bioassay for detecting an *erbB*-3 antigen in a biological sample comprising the steps of:

i) contacting said sample with an antibody according to claim 17, under conditions such that a specific complex of said antibody and said antigen can be formed; and

ii) determining the amount of said antibody present as said complexes.

19. A method for targeting a therapeutic drug to cells having high levels of *erbB*-3 receptors, comprising the steps of:

i) conjugating an antibody according to claim 17, or an active fragment thereof, to said drug; and

ii) administering the resulting conjugate to an individual with cells having high levels of *erbB*-3 receptors in an effective amount and by an effective route such that said antibody is able to bind to said receptor on said cells.

20. Use of the antibody of claim 17, or an active fragment thereof, conjugated to a therapeutic drug to target said therapeutic drug to cells having high levels of *erb*B-3 receptors.

FIGURE 1A FIGURE 1B

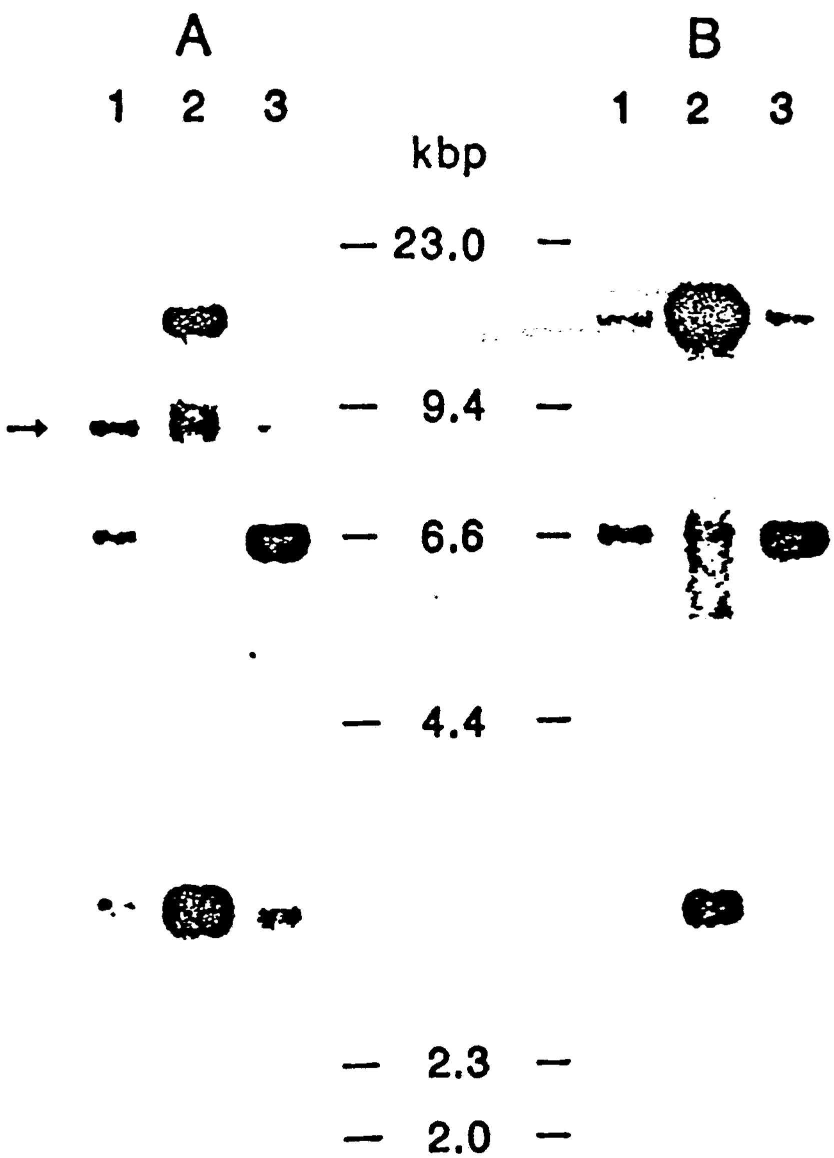

Figure 2

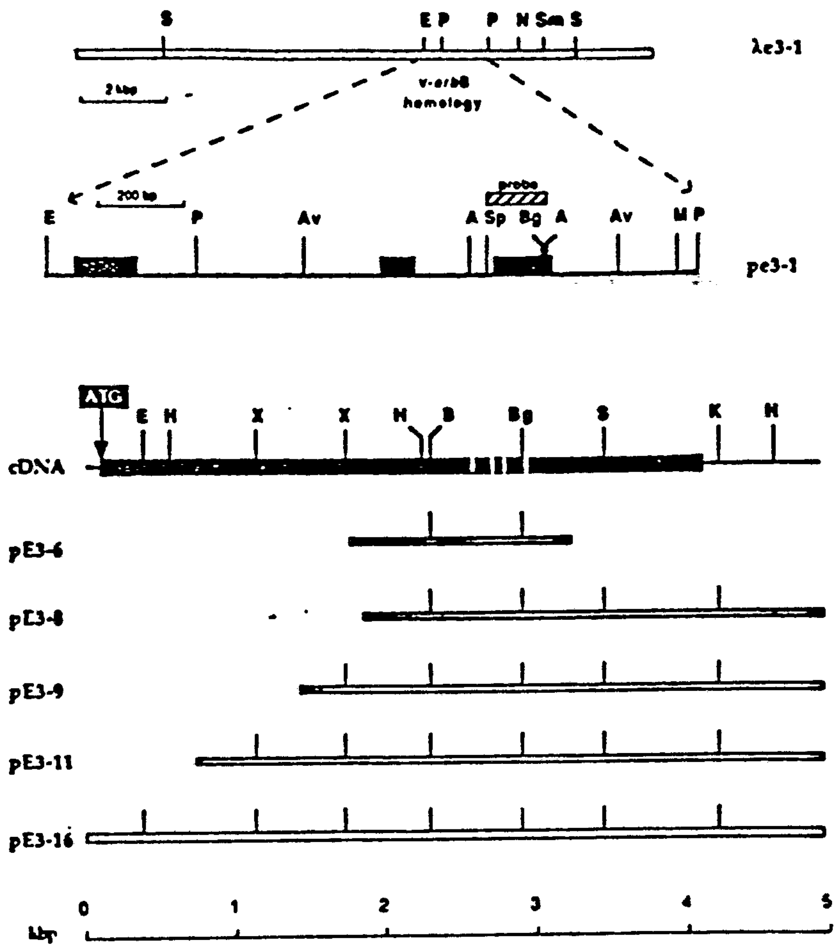
S
E P
P N Sm S
λe3-1
2 kbp
v-erbB
homology
200 bp
probe
E
P
Av
A Sp Bg A
Av
M P
pe3-1
ATG
E H
X
X
H B
Bg
S
K
H
cDNA
pE3-6
pE3-8
pE3-9
pE3-11
pE3-16
0
1
2
3
4
5
kbp

Figure 3

GAATTCCAGATCTCAGTGACTGATTCCCCCAACCTTAAGAATACTTTCTTCCCCTATACCTACAG

Gly Met Tyr Tyr Leu Glu Glu His Gly Met Val His Arg Asn Leu Ala Ala
GGA ATG TAC TAC CTT GAG GAA CAT GGT ATG GTG CAT AGA AAC CTG GCT GCC

Arg Asn Val Leu Leu Lys Ser Pro Ser Gln Val Gln Val Ala Asp Phe Gly
CGA AAC GTG CTA CTC AAG TCA CCC AGT CAG GTT CAG GTG GCA GAT TTT GGT

Val Ala Asp Leu Leu Pro Pro Asp Asp Lys Gln Leu Leu Tyr Ser Glu Ala
GTG GCT GAC CTG CTG CCT CCT GAT GAT AAG CAG CTG CTA TAC AGT GAG GCC

Lys
AAG GTGAGGAGACACAAAGGGTAAGGAGGCGGGGTGGAGTGAAGCATGGGGATAGGGAGCAGCCA

GTGGTCTCTTCCAGAGGCAAGCAGATGCTTCATGGTAAGTTCAAGGAGAGAAGGCTGCAGATGCCAG
ATATTTTAGTTCAGAGGGCAACAAAGAAAATAATGATCAAGAACTTGGGACTGGCCGGGCGCGGTGG
CTCACGCCTGTAATCCCAACACTTCGGGAGGCCAAGGCGGGTGGATCACAAGGTCAGGAGATCAAGA
CCATCCTGGCTAGCACGGTGAAACCCCGTCTCTACTAAATATACAAAAAAAAAAAAATTAGCCAGGC
GTGGCGGCATGCATCTGTACTCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATGGCGTGAACCCAGG
AGGCGGAGCTTGCAGTGGGCCGAGATCGCACCACTGCACTCCAGTCTGGGCGACAGAGCGAGACTCC
GTCTCAAAAAAAAAAAAAAAAGAATTTGGGACTTGGAAATCCTAAGAAAATTTGTGGAAATAAACTT

Thr Pro Ile Lys Trp Met Ala Leu Glu Ser
GTGATACCTCTATCTTTAATCCGCAG ACT CCA ATT AAG TGG ATG GCC CTT GAG AGT

Ile His Phe Gly Lys Tyr Thr His Gln Ser Asp Val Trp Ser Tyr G
ATC CAC TTT GGG AAA TAC ACA CAC CAG AGT GAT GTC TGG AGC TAT G GTCAG

TGCATCTGGATGCCCTCTCTACCATCACTGGCCCCAGTTTCAAATTTACCTTTTGAGAGCCCCCTCT
TAGAATCTCTAAGCACTTCAGATTTTTGTGTTAGATCAGGTTCTGCCTTCCCTTCACTTCATGCCCA

ly Val Thr Val Trp Glu
TGTCTACTATTTTGCCAGTGACTAGTCCATGTCTTCCTGCAACAG GT GTG ACA GTT TGG GAG

Leu Met Thr Phe Gly Ala Glu Pro Tyr Ala Gly Leu Arg Leu Ala Glu Val
TTG ATG ACC TTC GGG GCA GAG CCC TAT GCA GGG CTA CGA TTG GCT GAA GTA

Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Ala Gln Pro Gln Ile Cys Thr
CCA GAC CTG CTA GAG AAG GGG GAG CGG TTG GCA CAG CCC CAG ATC TGC ACA

Ile Asp Val Tyr Met Val Met Val Lys
ATT GAT GTC TAC ATG GTG ATG GTC AAG T GTGAGTTACCTGCTGAGCCCAACCATTTT

CTCTTTTTTCTTTTTTTTTCTTTTTTTTTTTTTTTGAGACAGAGTCTCACAATTGTCACCCAGGC
TGGAGTGCAATGGTGCAATCAATCTTGGCTCACTACAACCTCCGCCTCTCGGGTTCAAGAGATTCTC
CTGCTTCAGCTCCGGAGTAGCTGGGATTACAGCGCCCGCCACACCTGGATAACTGTTACACTTTTAG
TAGAGATGGGGTTTCACCATGTTGGCCAGGCTGGTCTCAAACTCCTGACCTCAGGTGATCCGCCTGC
CTCAGCTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCATCATGCTCGCCTGACTGCAG

Figure 4

[illegible]

Figure 5


```
1    MRANDALQVL GLLFSLARGS EVGNSQAVCP GTLNGLSVTG DAENQYQTLY KLYERCEVVM
61   GNLEIVLTGH NADLSFLQWI REVTGYVLVA MNEFSTLPLP NLRVVRGTQV YDGKFAIFVM
121  LNYNTNSSHA LRQLRLTQLT EILSGGVYIE KNDKLCHMDT IDWRDIVRDR DAEIVVKDNG
181  RSCPPCHEVC KGRCWGPGSE DCQTLTKTIC APQCNGHCFG PNPNQCCHDE CAGGCSGPQD
241  TDCFACRHFN DSGACVPRCP QPLVYNKLTF QLEPNPHTKY QYGGVCVASC PHNFVVDQTS
301  CVRACPPDKM EVDKNGLKMC EPCGGLCPKA CEGTGSGSRF QTVDSSNIDG FVNCTKILGN
361  LDFLITGLNG DPWHKIPALD PEKLNVFRTV REITGYLNIQ SWPPHMHNFS VFSNLTTIGG
421  RSLYNRGFSL LIMKNLNVTS LGFRSLKEIS AGRIYISANR QLCYHHSLNW TKVLRGPTEE
481  RLDIKHNRPR RDCVAEGKVC DPLCSSGGCW GPGPGQCLSC RNYSRGGVCV THCNFLNGEP
541  REFAHEAECF SCHPECQPME GTATCNGSGS DTCAQCAHFR DGPHCVSSCP HGVLGAKGPI
601  YKYPDVQNEC RPCHENCTQG CKGPELQDCL GQTLVLIGKT HLTMALTVIA GLVVIFMMLG
661  GTFLYWRGRR IQNKRAMRRY LERGESIEPL DPSEKANKVL ARIFKETELR KLKVLGSGVF
721  GTVHKGVWIP EGESIKIPVC IKVIEDKSGR QSFQAVTDHM LAIGSLDHAH IVRLLGLCPG
781  SSLQLVTQYL PLGSLLDHVR QHRGALGPQL LLNWGVQIAK GMYYLEEHGM VHRNLAARNV
841  LLKSPSQVQV ADFGVADLLP PDDKQLLYSE AKTPIKWMAL ESIHFGKYTH QSDVWSYGVT
901  VWELMTFGAE PYAGLRLAEV PDLLEKGERL AQPQICTIDV YMVMVKCWMI DENIRPTFKE
961  LANEFTRMAR DPPRYLVIKR ESGPGIAPGP EPHGLTNKKL EEVELEPELD LDLDLEAEED
1021 NLATTTLGSA LSLPVGTLNR PRGSQSLLSP SSGYMPMNQG NLGESCQESA VSGSSERCPR
1081 PVSLHPMPRG CLASESSEGH VTGSEAELQE KVSMCRSRSR SRSPRPRGDS AYHSQRHSLL
1141 TPVTPLSPPG LEEEDVNGYV MPDTHLKGTP SSREGTLSSV GLSSVLGTEE EDEDEEYEYM
1201 NRRRRHSPPH PPRPSSLEEL GYEYMDVGSD LSASLGSTQS CPLHPVPIMP TAGTTPDEDY
1261 EYMNRQRDGG GPGGDYAAMG ACPASEQGYE EMRAFQGPGH QAPHVHYARL KTLRSLEATD
1321 SAFDNPDYWH SRLFPKANAQ RT
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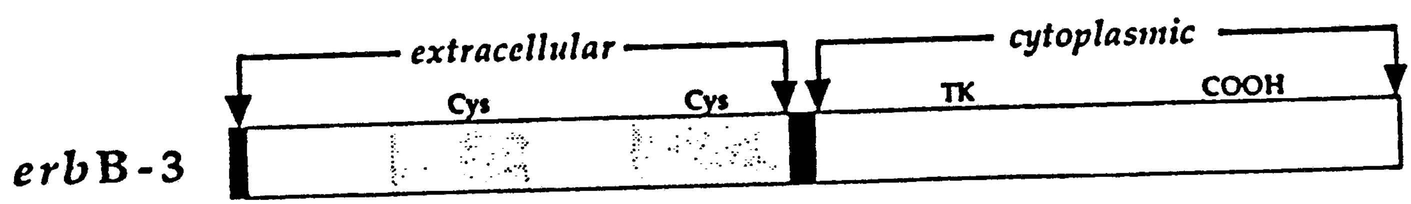


	Cys	TK	COOH
*erb*B-2	43	62	-
*erb*B/EGFR	45	60	-
met	-	32	-
eph	-	32	-
IR	22	29	-
fms/CSF1R	-	27	-

% IDENTITY

Figure 6

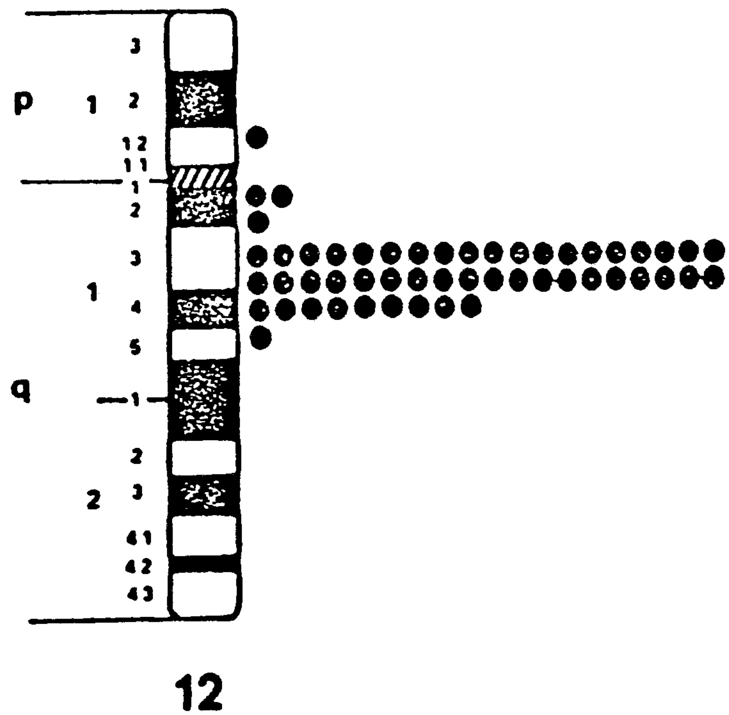


FIGURE 7A FIGURE 7B

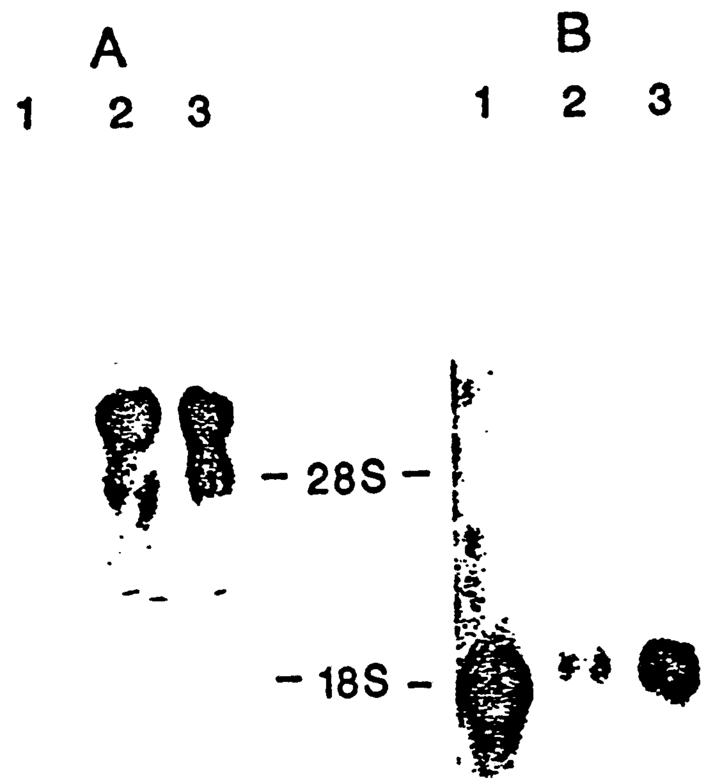
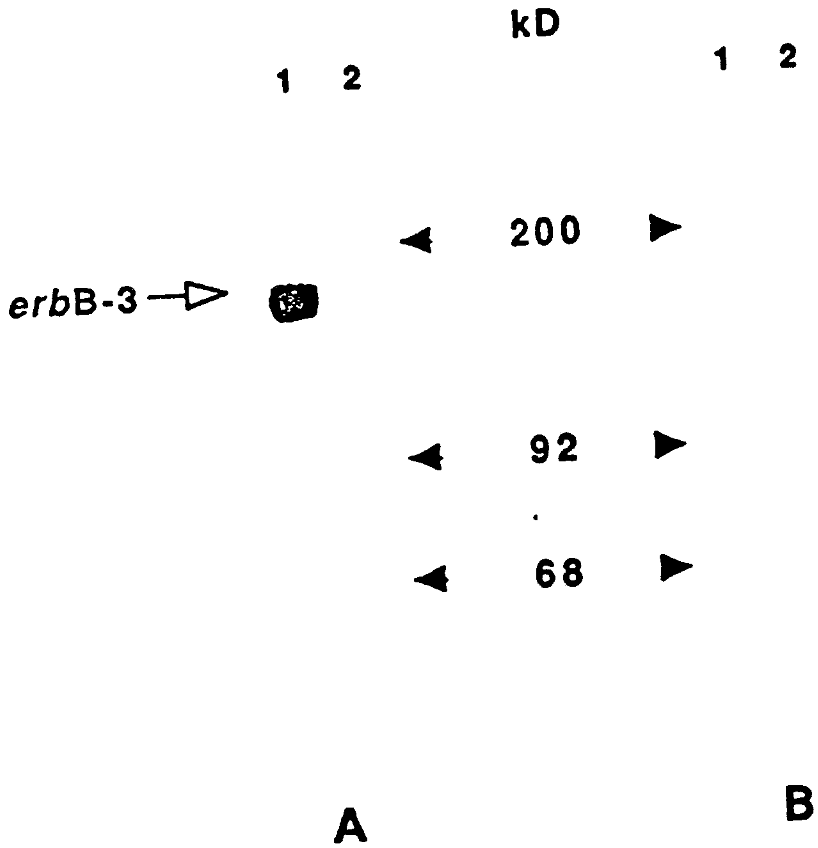


FIGURE 8A FIGURE 8B



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/07025

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5):C07H 15/12; C12Q 1/68; G01N 33/566; A61K 35/14, 39/42
U.S.CL.:536/26,27,28,29; 435/6, 243 ; 436/501; 530/387;424/85.91,86

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/26, 27, 28, 29; 435/6, 243; 436/501,63, 94; 530/387; 424/85.91, 86; 935/66, 71, 77, 78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS: antibody/carrier/therap? and sequence searches, Figures 3-4.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,Y	Proceedings of the National Academy of Sciences, Vol. 86, issued December 1989, Kraus et al., "Isolation and Characterization of ERBB3, A Third Member of the ERBB/Epidermal Growth Factor Receptor Family: Evidence for Overexpression in a Subset of Human Mammary Tumors", pages 9193-9197, see pages 9193-9196.	1-15
Y	Sciences, Vol. 229, issued 06 September 1985, King et al., "Amplification of a Novel v-erbB -Related Gene in a Human Mammary Carcinoma", pages 974-978, see pages 974-975.	1-15
Y	Science, Vol. 230, issued 06 December 1985, Coussens et al., "Tyrosine Kinase Receptor with Extensive Homology to EGF Receptor Shares Chromosomal Location with neu Oncogene", pages 1132-1139, see page 1133.	1-15

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 February 1991	28 MAR 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Stephanie W. Zitomer, Ph.D Stephanie Zitomer ebw

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Nature, Vol. 319, issued 16 January 1986, Yamamoto et al., "Similarity of Protein Encoded by the Human c-erbB-2 Gene to Epidermal Growth Factor Receptor", pages 230-234, see pages 231-232.	1-15
Y	Science, Vol. 237, issued 10 July 1987, DiFiore et al., "erbB-2 Is a Potent Oncogene When Overexpressed in NIH/3T3 cells", pages 178-182, see pages 179-180.	16-18
Y	US, A, 4,867,973 (Goers et al.) 19 September 1989, see columns 45-46.	19-20

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers___________, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See Attached Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

PCT/US90/07025

Attachment to PCT/ISA/210 (PART VI)

OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claims 1-10, 12, 13, 15, comprises a first product the erbB-3 gene and a first process of using in a hybridization assay;

Group II, claims 11, 14, 16, comprises a second product, a polypeptide encoded by the erbB-3 gene and a second process of using in a ligand assay;

Group III, claims 17, 18 comprises a third product, an antibody to said polypeptide and a third process of using in a (third) assay;

Group IV, claims 19, 20, comprises a fourth process of using the antibody for therapy.